REMARKS

Claims 68 and 70-80 are in this application. Claims 1-67, 69 and 81 have been cancelled.

Claims 68, 73-77 and 80 have been amended. Claims 68 and 80 have been amended to include the subject matter of claims 69 and 81, respectively.

Claims 73-77 have been amended to replace astringent, anesthetic, protectant, wound healing agent and keratolytic with astringents, anesthetics, protectants, wound healing agents and keratolytics, respectively.

Claim 79 has been amended to delete the word "dry" in step c) of the claim. Step f) of claim 79 has also been amended to include examples of organic solvents of medium polarity. Support for this amendment is found on page 14, lines 14-16 of the specification.

The amendments to claims 73 and 80 obviate the objections to these claims.

In view of the amendments to claims 73-79 it is respectfully requested that the rejection of these claims under 35 USC 112, second paragraph be withdrawn.

The Examiner asserts that claims 68-81 are vague and indefinite due to the phrase "containing flavonoids and phenolic compounds. This is respectfully traversed.

The naturally occurring plant *Euphorbia prostrata* is known to contain flavonoidal and phenolic compounds and ther constituents. See Anil K. Singla, et al. Journal of Ethnopharmacology, 1990, 29, page 291 and Takashi Yoshida et al., Chem. Pharm, Bull., 1994, 42, page 2005 (attached). As described in the specification flavonoidal and phenolic compounds are the main components of the extract of *Euphorbia prostrate,* see

for example, page 4, line 13-page 5, line 7. However, none of the prior art discloses any composition or any process of making a composition comprising an extract of *Euphorbia prostrata* wherein different specific flavonoids and phenolic compounds are present in the amounts specified in the claims to provide the desired therapeutic effect as claimed in the present invention.

It is submitted that all of the objections and rejections have been overcome.

It is submitted that the application is in condition for allowance and favorable consideration is respectfully requested.

Respectfully submitted,

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Chem. Pharm. Bull. 42(10) 2005—2010 (1994)

Tannins and Related Polyphenols of Euphorbiaceous Plants. XII.[1] Euphorbins G and H, New Dimeric Hydrolyzable Tannins from *Euphorbia prostrata* and *Euphorbia makinoi*

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Received March 15, 1994; accepted July 1, 1994

Two new ellagitannin dimers, euphorbins G (20) and H (21), together with 12 known polyphenols, were isolated from the leaves of *Euphorbia prostrata* (*E. chamaesyce*). Their structures, having $^1C_4$ and $^4C_1$ glucopyranose cores in each molecule, were established by spectroscopic and chemical methods. These new dimers, and 13 known hydrolyzable tannins, among which six are the same as those from *E. prostrata*, were also isolated from *E. makinoi*.

Keywords *Euphorbia prostrata*; *Euphorbia makinoi*; Euphorbiaceae; tannin; euphorbin G; euphorbin H

In a previous study on the tannins of euphorbiaceous plants, we isolated and chemically characterized euphorbins A—F, dimeric hydrolyzable tannins of a new class having a geraniin moiety as a monomeric unit, from *Euphorbia hirta* L.[2] and *Euphorbia tirucalli* L.[3] We also isolated a new dimer, euprostin B, from *Euphorbia prostrata* AIT., collected in Fujian, China, together with rugosins D, F and G, which are oligomers of a type different from that of euphorbins.[4] During the survey of the tannins in the *Euphorbia* species, we found that *E. prostrata* (*E. chamaesyce* L.) collected in Okayama, Japan, shows different pattern in HPLC from that of the species collected in China. The present paper describes the isolation and structural elucidation of two additional members of euphorbin-type dimers, named euphorbins G and H from *E. prostrata* collected in Okayama. These new dimers were also obtained from *E. makinoi* HAYATA together with several known tannins which are the same as those from *E. prostrata*.

The aqueous acetone homogenate of the dried leaves of *E. prostrata* was extracted successively with ether, EtOAc and n-BuOH. The EtOAc extract was chromatographed over Toyopearl HW-40 and/or MCI-gel CHP 20P to yield the new tannin, euphorbin G (20), and nine known compounds. Among them, two were identified as [illegible] (1) and isoquercitrin (2), and the other seven were characterized as 2-O-galloyl-4,6-(S)-hexahydroxydiphenoyl-glucose (3),[5] strictinin (4),[6] tellimagrandin [illegible] (6),[6] corilagin (8),[7] geraniin (9),[7] and [illegible] F (11), by comparison of their physical data with [illegible]. Similarly, the n-BuOH extract [illegible] (20) and H (21), 5, pedunculagin [illegible] (12),[8] and euphorbelin (13).[11]

[illegible] portion of the aqueous acetone [illegible] the dried aerial parts of *E. makinoi* [illegible] in an analogous way to give [illegible] (20) and H (21), along with 3, 5, 7, 8, 9, 12, [illegible], furosin (18),[14] and mallotusinic acid [illegible] tri-O-galloyl-β-D-glucose (14),[11] 1,2,4,6-[illegible]-β-D-glucose (15),[11] 1,2,3,4,6-penta-O-[illegible] glucose (16),[11] chebulagic acid (10)[13] and 9 [illegible] obtained similarly from the EtOAc-soluble portion.

Euphorbin G (20) and H (21) were suggested to be dimeric hydrolyzable tannins by positive color reactions with $FeCl_3$ and HOAc–$NaNO_2$ reagents[10] on a TLC plate, and by their large retention volume on normal-phase HPLC,[1] both of which are similar to those of 11 and 12. The dimeric nature of euphorbin G was also supported by the FAB-MS ion peak at m/z 19[illegible] ascribable to (M+Na)$^+$. Acid hydrolysis of 20 with hot 5% H_2SO_4 yielded glucose as well as gallic acid, ellagic acid and valoneic acid dilactone, which were identified after methylation producing 27—29. The ^{1}H-NMR spectrum of 20 showed signals assignable to three galloyl groups and two pairs of one-proton singlets ascribable to a hexahydroxydiphenoyl (HHDP) group and a valoneoyl unit in the aromatic region. The pairs of methine proton signals [δ 5.12 (s) and 4.87 (d, J=1.5 Hz), H-1 [illegible]] proton signals [δ 6.48 (s) and 6.20 (d, J=1.5 Hz), H-[illegible]] and aromatic proton signals [δ 7.22 (s) and 7.12 (s), H-[illegible]] are characteristic of a dehydrohexahydroxydiphenoyl (DHHDP) group existing as an equilibrium mixture of six- and five-membered hemiacetal forms, as found in the geraniin (9) molecule.[7] Duplication of the signals were also observed for the sugar proton signals (Table I) and some other signals, and was thus attributed to the presence of a DHHDP group in 20. The paired signals due to the DHHDP group are also exhibited in the ^{13}C-NMR spectrum of 20, by the signals of an α,β-unsaturated ketone system [δ 192.0, 195.0 (C-4'''), 154.1, 149.3 (C-[illegible]), 125.[illegible] (C-[illegible])] and methine carbon signals [δ 46.6 and 52.0 (H-1 [illegible])].

Upon condensation with o-phenylenediamine in an acidic medium, 20 gave a phenazine derivative (22). Its ^{1}H-NMR spectrum, which is simplified by the absence of duplication of peaks, clearly indicated the presence of an HHDP and a valoneoyl group [δ 7.12, 6.97, 6.65, 6.64, 6.22 (each 1H, s)] in addition to a phenazine [δ 8.31, 7.49 (1H each, s) and 7.99 (2H, m), 8.32, 8.20 (1H each, br d, J=9 Hz)] and three galloyl [δ 7.01 (2H, s), 6.95 (4H, s)] units. The sugar proton signals and the aromatic proton signals shown above are similar to those of the phenazine derivative (24)[3] from euphorbin F (23), except for the

Journal of Ethnopharmacology, 29 (1990) 291–294
Elsevier Scientific Publishers Ireland Ltd.

TOPICAL ANTIINFLAMMATORY EFFECTS OF *EUPHORBIA PROSTRATA* ON CARRAGEENAN-INDUCED FOOTPAD OEDEMA IN MICE

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(Accepted January 18, 1990)

Summary

The ethyl acetate extract and a fraction, KSE-23, isolated chromatographically from the ethyl acetate extract of *Euphorbia prostrata*, showed significant antiinflammatory activity when topically applied in a murine model of carrageenan footpad oedema. KSE-23 was found to be more potent than indomethacin given in the same manner.

Introduction

The ethyl acetate extractive of the entire plant of *Euphorbia prostrata* Ait. (Euphorbiaceae) containing flavonoids and their glycosides (apigenin and luteolin as the main constituents) and a fraction, KSE-23, obtained from ethyl acetate extract have been shown to have significant antiinflammatory activity (AIA) in rats on oral administration (Singla and pathak, 1989). KSE-23 has been identified as a mixture of 55.8% apigenin-7-galactoside and 44.2% luteolin-7-galactoside (unpublished data). Flavonoids such as apigenin and luteolin are known to possess marked AIA on topical application, the potency being similar to indomethacin (Della Loggia et al., 1986a). In the present study, the topical AIA of the ethyl acetate extract and KSE-23 were investigated using murine carrageenan footpad oedema, a widely accepted model of acute exudative inflammation.

Materials and methods

Materials

The ethyl acetate extractive of *Euphorbia prostrata* and the fraction labelled as KSE-23 were prepared following our previously published procedures of extraction and chromatographic purification (Singla and Pathak, 1989).

0378-8741/90/$01.75